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TRIALS OF SEVERAL DENSITY ESTIMATORS ON A BUTTERFLY POPULATION

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INTRODUCTION

The density of an animal population is notoriously difficult to estimate, and new methods are consequently being developed continually. Since some newer procedures have been tried little in the field, our objective was to compare several of them to several older ones.

Some of the extensive literature on population estimation has been reviewed by Hanson (1967), Southwood (1966), and Ricker (1958), making further discussion of the theory not now warranted. The book by Southwood emphasizes entomological applications, and especially how to obtain reliable data. It bears repeating that workers have found it considerably easier to develop the mathematical bases of the estimating techniques than they have to solve the biological and economic problems of getting unbiased data in adequate amounts for use in the estimators.

To compare the estimating procedures, we required to study: (1) a natural population, (2) one that was fairly dense, (3) a relatively isolated population, to reduce egress of marked animals, (4) yet one comprising a highly mobile species, and finally, (5) a population that could be found close at hand and approached and captured with a minimum of problems. For these purposes, the common alfalfa butterfly (*Colias eurytheme*) turned out to be very good. The habitat and behavior of the alfalfa butterfly, among other matters, were discussed by Hovanitz (1948).

A suitable population of the butterflies was found in a field of alfalfa (*Medicago sativa*) located on an experimental farm of California State College at Pomona. The field contained 14.2 acres, was rectangular in shape, and was surrounded by grass, fallow land, and an orange grove. It appeared to be well isolated from other areas providing habitat for *Colias eurytheme*. The alfalfa was somewhat thinly planted and averaged about 12-14 inches tall. The field data were collected on three consecutive days, August 13 through 15, 1964. (Further work was attempted in another alfalfa field in August of 1966 but inadequate isolation of the population precluded any reliance on marking methods.)

METHODS OF GETTING DATA

Throughout the field, two or more workers moved about at random, netting the butterflies that came within reach. Upon capture, each butterfly was marked with a spot of nail polish on the ventral, distal surface of the wing; the butterfly was held for a few moments to allow the paint to dry and then was released, in the manner to be described in a forthcoming paper by Hovanitz. By using several dots, it was easily possible to show how many times a given individual butterfly had been captured. Marking was not continued beyond the second day.

Concurrently with this effort, in a second "experiment," two other workers attempted to make total counts on sample plots in the Cal Poly field. Before our work began, the alfalfa field had been divided lengthwise into 10 strips, each about 93 feet wide, by low dikes erected to keep irrigation water in place. As the observers moved lengthwise along each resulting strip, they walked 20 long steps (about 60 feet) and counted all butterflies within the resulting "plots," then stopped and recorded the insects seen, and continued to repeat this process. The size of the plots (60 x 93 feet) was determined partly by the fact that the observers concluded not to count any butterflies that were more than 60 feet beyond them.

In another experiment, a series of cursory, incomplete counts was made in this alfalfa field by one observer. In this case the observer walked rapidly back and forth across the field from one side to the other. The beginning point and ending point of each walk were guided on a stake previously set at the middle of each side. When he crossed the field, the observer's eyes were fixed straight ahead on the stake located at the far side, but all *Colias* that could be seen within the arc encompassed by the observer's vision as he looked straight ahead were included in the counts. Since the field was crossed 35 times, 35 superficial samples of butterflies were gathered.

FREQUENCY OF CAPTURE

The repeated capturing, marking, and releasing of the butterflies produced a frequency distribution in which f_1 butterflies were caught x times, f_2 were caught 2x times, and so on up through f_i animals taken x_i times for each of the two days on the Cal Poly field, as is shown in Table 1. (The estimated abundances of butterflies according to this and all other methods is shown in Table 2.) The resulting data were used to estimate the frequency of the animals seen zero times, i. e., to estimate the missing class f_0 in the truncated distribution. After the number of unseen animals was estimated, obviously it could be added to the number of those actually seen to give the estimated total number of butterflies in the whole population.

As was discussed in the earlier review (Hanson, 1967), several papers give promising procedures for estimating the total abundance, K, of the population from such frequency of capture data. Among these, the paper by Craig (1953) contained a refined version of a moment model using data obtained by Hovanitz (Method 2 of Craig's paper), which required the data to have an underlying Poisson distribution; this model was tried on the data shown in Table 1. A paper by Edwards and Eberhardt (1967) contained several estimating procedures, among which was the maximum-likelihood model requiring data coming from a geometric distribution.

A further procedure mentioned by Edwards and Eberhardt involved plotting of the capture frequencies on semi-log paper. It is well known that when a regression relationship is curvilinear, it can often be transformed into a linear one by plotting the logarithm of one or both variables (see, for example, Bailey, 1959:94). In the familiar expression for the linear regression line

$$Y = a + b X,$$

Y is the dependent variable; X is the independent variable; a is the height on the Y axis where the line began, and b is the slope of the line. One can plot the number of animals captured once against the number 1, the number captured twice against the number 2, etc. When semilog paper is used and the number of animals is plotted on the logarithmic scale (i.e., on the Y axis) and the number of times that they were captured is plotted on the equal-interval scale (i.e., on the X axis) a straight line may result. If the points (X,Y) result in a straight line, then the transformed statement of the regression equation must have finally ended up with the form (see Steel and Torrie, 1960:334):

$$\log Y = a + bX.$$

TABLE 1

The Number of Times That Butterflies (*Colias eurytheme*) Were Captured and Marked in an Alfalfa Field Near Pomona, California

	x (Number of Captures Per Individual)	f (Frequency)	xf	x^2f
I. ALL SEXES:				
	0	---	---	---
	1	81	81	81
	2	35	70	140
	3	11	33	99
	4	1	4	16
Sum:	---	128	188	336
II. FEMALES ONLY:				
	0	---	---	---
	1	46	46	46
	2	24	48	96
	3	10	30	90
	4	1	4	16
Sum:	---	81	128	248

However, the last equation differs from the expression used by Edwards and Eberhardt (1967:92), which they got from the geometric-distribution model, since their expression had the form

$$\log Y = a + X \log b \ .$$

Therefore, it seems doubtful that plotting the capture frequencies on semilog paper, and the corresponding number of times each animal was captured on the equal-interval scale, will preserve the meaning of the geometric expression, but statisticians should investigate the matter further. In any case, the plotting of our data on semilog paper gave good estimates, as will be shown later (Figure 1).

Poisson Estimator

The data on captures of *Colias eurytheme* for use in the Poisson estimator shown as Method 2 in Craig, 1953) are given in Table 1, and the estimated number obtained by all methods are summarized in Table 3. Based on the data for all sexes, the result for Method 2 gave

$$\hat{K} = 188^2 \ / \ (336 - 188) = 239.$$

When the error is expressed as a decimal fraction of the estimated mean according to Craig's formula the result at the 95% confidence level is:

$$\text{Standard Error} = \sigma^2_{n/n} = 2 \quad (239) \ / \ 188^2 = .1162.$$

Therefore, the confidence limits became (Table 3)

$$185 < 239 < 293 \ .$$

When only the data for females were used, the estimated number of females was 137 and its 95% confidence limits were 102 to 172. Because the sex ratio among butterflies seems usually to be approximately unity, these numbers can be doubled to give K = 274 and a confidence interval extending from 204 to 344 (Table 3).

As work progressed, some marked butterflies moved out of the field, and the population started declining. We suspected that the insects moving out were, as usual, mainly males, which made the results based on females better than that based on both sexes combined. Although the real number of butterflies inhabiting the field when work began was obviously unknown, the results show that it was approximately 275, and the 95% confidence limits extended from 200 to 350.

Evidently the data did come from a Poisson distribution or from one that approximated it tolerably well. The procedure required that $\underline{p}$ not change much from trial to trial, and evidently

TABLE 2

The Number of Times That Free-Ranging Butterflies (*Colias eurytheme*)

Were Observed on Sample Plots in an Alfalfa Field Near Pomona, California

x (Number of *Colias* Seen Per Plot)	f (Frequency of Plots)	fx (Total *Colias*)	fx^2
0	60	0	0
1	27	27	27
2	16	32	64
3	4	12	36
4	4	16	64
Sum: ---	111	87	191

Variance = s^2 = 1.116

Mean = $\bar{x}$ = .784

this condition was met. The labor of capturing the butterflies during the hot weather was considerable, yet was small compared to that required to catch and mark animals such as fishes, birds, and mammals with nets, baited traps, or comparable means.

In summary, the frequency-of-capture method using the Poisson model gave good results. Movement of marked butterflies off the study area was a problem, just as it is for mark-and-recapture models or removal models (see Ricker, 1958:86 for further discussion), making it necessary to work quickly and to stop as soon as a few of the animals have been captured as many as four times.

Geometric Estimator

When the same basic data (Table 1) were used in the equation of Edwards and Eberhardt (1967), the results were

$$K = \frac{128}{1 - (128 - 188)} = 401$$

Confidence limits were not calculated since no procedure for this was given by Edwards and Eberhardt.

The estimate of 401 butterflies obtained from the geometric model was well above what we believed to be approximately the correct upper bound of 350. Why this model did not give as good an estimate as the Poisson was not clear, but possibly it was because the geometric model is more suitable for contagious (clumped) spatial distributions. However, these butterflies flew about in an apparently random manner, and gave no evidence of significant aggregation.

Regression Estimator

When the frequencies-of-capture were plotted on semi-log paper, the resulting points fell remarkably close to a straight line (Figure 1). For both sexes combined, the fit was very good except for the class of four captures per individual, where the sample size was, of course, very small. When this point was ignored, the plotted line indicated that the zero class of frequencies was about 162, and that the total population was thus about 290 (Table 3). For females only, the fit of the line was even better (Figure 1), and it indicated that about 234 animals were not captured, making the total population about 315 (Table 2). Both estimates are near what was believed to be the true number, 275.

Since the method showed promise and could be applied quickly, it should be tested considerably more. Getting the

TABLE 3

Summary of Estimates of the Number of Butterflies (<u>Colias</u> <u>eurytheme</u>) Occurring in an Alfalfa Field Near Pomona, California

Method	k	95 Confidence Limits	
I. FREQUENCY OF CAPTURE			
1. Poisson, Both Sexes, 1st Day.	239	185	293
2. Same as Preceding, Except: 2nd Day	46	Not Calculated	
3. Same as Preceding, Except: Based on Females Only, 1st Day; Results Were Doubled (to Include Males).	274	204	44
4. Frequency of Capture - Geometric Model, Both Sexes, 1st Day.	401	No Procedure Available	
5. Same as Preceding, Except: Based on Females Only; Results Were Doubled (to Include Males).	442	No Procedure Available	
6. Frequency of Capture - Regression Method, Both Sexes, 1st Day.	290	Not Calculated	
7. Same as Preceding, Except: Based on Females Only, 1st Day; Results Were Doubled (to Include Males).	315	Not Calculated	
II. TOTAL COUNTS ON SAMPLE PLOTS			
1. 1st Day	87	65	109
2. Same as Preceding, Except: 2nd Day.	102	Not Calculated	
III. RELATION OF VARIANCE TO MEAN			
1. Cursory Counts, 111 Plots Occurring in 8 Rows, 1st Day.	0	Not Calculated	
2. Same as Preceding, Except: 2nd Day.	0	Not Calculated	
3. Same as Preceding, Except: Based on Sum of Each of 8 Rows; 1st Day.	664	21	
4. Same as Preceding, Except: Data for Both Days Combined (n = 16 Rows).	0	Not Calculated	
5. Same as Preceding, Except: Whole Field Subject to Scanning, 3rd Day.	0	Not Calculated	
IV. MARK-RATIO MODEL			
1. The Dahl, or Petersen Method, Data from Both Days	307	210	610
V. REMOVAL METHOD			
1. Data from 1st Day.	187	0	402

data for plotting the regression line is obviously subject to all of the problems affecting other methods based on marked animals (see Ricker, 1958:86-100; Hanson, 1967).

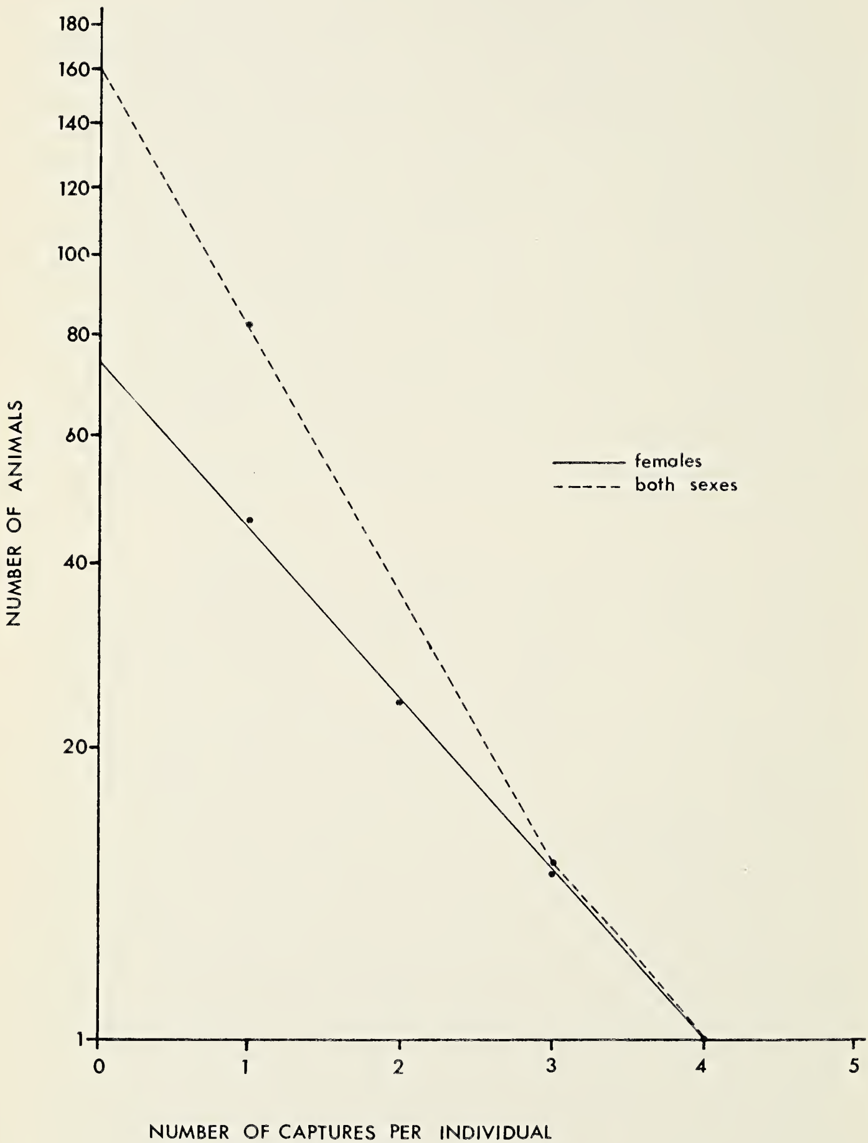
TOTAL COUNTS ON SAMPLE PLOTS

On the first day 87 animals were counted and on the second day two counts yielded, respectively, 87 animals (Table 3) and 102 animals. These figures were undoubtedly much too low, mainly because resting butterflies were tending to fly off the plots before the observers could determine whether the butterflies were within a plot's boundaries. Also, the relatively rapid and erratic flight paths of moving butterflies made it difficult to tell when they were above a given plot and the observers erred on the conservative side. Since the confidence limits for the first day's estimate were rather narrow (Table 3), the bias appeared to be rather consistent. The method of total counts could be made more useful by (a) marking off the boundaries in a more elaborate, easily-recognized way than was done here and (b) by enlarging the plots; but we believe that, for highly mobile animals such as *Colias eurytheme*, the plotless, frequency-of-capture methods are superior when ingress and egress are not important problems.

RELATION OF VARIANCE TO MEAN

This method is described (Hanson and Chapman, in press; Hanson, 1967) as a method for rapidly estimating the number of groups of free-ranging animals from cursory, incomplete counts. None of the animals need be marked or removed, and total counts of any component are not required; but, on the other hand, the model requires (in addition to the usual random sampling) that the data come from a binomial distribution. Although individual animals usually tend to be clumped spatially, the groups themselves should be distributed more at random, leading to a binomial distribution of groups. Therefore, the model deals only with groups. After the worker estimates the total number of groups, he would of course multiply by the average group size to get total population. Since the alfalfa butterflies were here essentially solitary, except for some very brief liaisons between copulating individuals, it turned out that group size was usually 1. However, as is indicated by the estimates shown in Table 3, the proper data could not be obtained.

The data on the counts of individuals seen per plot and the resulting variance and mean per plot are shown in Table 2. When the data were substituted in the proper formula the results gave



180
160
140
120
100
80
60
40
20
1
NUMBER OF ANIMALS
0
1
2
3
4
5
NUMBER OF CAPTURES PER INDIVIDUAL
females
both sexes

$$K = \frac{.784^2}{.784 - 1.116} = O$$

Since the variance exceeded the mean, this caused a negative estimate, interpreted biologically as a population of size zero (Table 3).

When the preceding samples of Table 2 were combined within each of the 8 transects to smooth out random error, 8 samples of butterflies were obtained: 8, 17, 11, 7, 11, 12, 13, 8. For this series, the mean was 10.875 and the variance was 10.697, leading to the following estimate of the total population (Table 3):

$$\hat{K} = 10.875^2 \ / \ (10.875 - 10.697) = \text{ca. } 664.$$

The 90% confidence limits were obtained from Dr. Chapman's equations (Hanson and Chapman, in press)

$$1 - \frac{(7)\ (10.697)}{(10.875)\ (2.17)} < p < 1 - \frac{(7)\ (10.697)}{(10.875)\ (14.1)}$$

where 2.17 and 14.1 are the upper and lower values of Chi-square, for 7 d.f. and .95 and .5 probability, respectively, read off from a table such as that of Fisher and Yates (1957:45). After the indicated arithmetic is performed, it resulted in

$$1 - 3.17 < p < 1 - .488 \ .$$

Since a negative value of p in this double inequality (on the left alone) is biologically impossible, the lower bound could not be less than 0, and the confidence interval for the probability of seeing a given animal became

$$0 < p < .512 \ .$$

The confidence limits for K finally became 10.875 / .512 = 21.24; and 10.875 / 0, which can be taken as infinity.

All other attempts to estimate K from the relation of variance to the mean failed because the variance was too high. Evidently (a) the true population density varied greatly from one plot to the other or (b) the animals were aggregated into larger groupings that were not recognized as such, or (c) the activities of the observer introduced considerable extraneous variation. Most likely each problem occurred to a degree.

First, the outside transect on each side of the field appeared to continually have fewer butterflies than did the inner transects; why the butterflies tended to use the outside parts of the field less, was not clear, but superficially the alfalfa appeared thinner there.

Second, at times the butterflies were momentarily aggregated a female, but these groups were treated as chance events and the

Colias in them were recorded as individuals (i.e., several "groups" containing one animal each).

Third, the principal cause of the excessive variation seemed to be the lack of an objective method for determining the boundaries of the area scanned and whether or not observed butterflies were within those boundaries during the rapid, cursory counts. Since the estimator based on relations of the mean to the variance would provide an easy and rapid way of estimating density if the proper data can be obtained, it is important to find an objective way to make the counts.

MARK-RATIO MODEL

The well-known mark-and-recapture method, apparently first used on animals by Dahl (1917), and reviewed extensively by Ricker (1958), Southwood (1966), and Chapman (1954), was tried here; for data we had 128 different butterflies caught the first day and 24 caught the second day, of which 10 had been marked at least once. Therefore,

$$\hat{K} = \frac{(128)\ (24)}{10} = 307\ ,$$

with limits (210, 610) (Table 3).

The small size of the sample caught on the second day, small in spite of considerable effort, indicated that much of this *Colias* population had left the field. Egress would cause no problem so long as the ratio of marked to unmarked animals did not change. Since there seemed to be no evidence that marked animals were leaving at a faster rate than the others, the estimate of 307 was reasonably close to the true population size. The confidence limits were somewhat wide, mainly because of the small sample in loose groups, perhaps due to attraction of several males to size collected on the second day.

REMOVAL METHOD

The removal method of population estimation was apparently begun by Hjort and Ottestad (1933) and has since been reviewed by several persons, including particularly Zippin (1956). In the present work it was expected that the count for the first day could be compared to that for the second day although no animals nor plots were removed. It was planned that any animal caught on the second day that bore a mark from the first day would be treated mathematically as dead. However, the decline in population size during the two days negated one of the main requirements for use of the removal models. As a result, another approach was tried.

For both sexes combined, 81 butterflies were caught once on the first day, and 35 were caught twice on that day (Table 1). Now let it be imagined that two independent samples had been taken on that day, each involving equal effort and the other standard assumptions of the removal method, and that in the first sample 81 animals were caught. If efforts, etc., were constant, then 81 should have been caught in the second (hypothetical) sample, of which 35 would have been carrying earlier marks. The 35 marked ones (Table 1) found in the second imaginary sample may be subtracted from the 81 assumed caught, leaving 46 as the size of the unmarked portion in the second sample. This manipulation provides the raw data for use in the estimating equation:

$$K = \frac{81^2}{81 - 46} = 187 .$$

Where c_1 and c_2 are the number of animals caught and removed on the first and second surveys, respectively, then the standard error of the estimate is (Zippin, 1956)

$$\frac{c_1^2\, c_2^2\, (c_1 + c_2)}{(c_1 - c_2)^4} = \frac{(6561)\ (2116)\ (127)}{1{,}500{,}625} = 108 .$$

Therefore, the upper and lower limits, at the 95% confidence level, were (0, 402) (Table 3).

The estimate of the total population size, 187, seemed too small, although the confidence limits included the most reasonable values, 275 to 300. The difficulty seemed to be that more unmarked animals should have appeared in the second sample, requiring that the number marked for the second time should have been smaller. Therefore, the possibility was present that once a butterfly was marked, it was more prone to be caught again, but if this were so, the estimate based on the Dahl mark-ratio method should have been smaller. The question was not definitely answered but "proness to capture" should not have caused much trouble.

DISCUSSION AND SUMMARY

How satisfactory any estimator of density does perform depends in part on each person's concept of what is "satisfactory." According to our experience, most zoologists expect results too close to the real population mean and often seem to think that an error much over 10-20% is excessive. Yet considering the many possible sources of error even in stationary populations such as plants, it is a wonder that a highly mobile animal group can have its density estimated within one order of magnitude. Certainly it appears that estimates on highly mobile animals should be considered reasonably good if they are within 50% of the true population size, although attempts should of course continue to be made to find better techniques.

Viewed in this light, several estimates obtained in the present study were fairly close to what seemed reasonable, that is about 275 to 300; frequency-of-capture models, based on either the Poisson distribution or on a regression line, and the mark-ratio model gave estimates near that value. Methods based on finding plot boundaries, such as the mean-variance model or total counts, were not as satisfactory, although they might become so when the plots are larger and better marked. At least 128 different butterflies were caught and marked, and the latter sets a known minimum limit for the population. The upper limits were either about 344 (frequency-of-capture, Poisson), 402 (removal method), or 610 (mark-ratio method) (Table 3). Which of these is better cannot be dogmatically stated, since the correct answer rests partly on a matter of intuition, and confidence level associated with the value selected. In our opinion, the true upper limit of the population estimate should have been not more than about 400, i.e, 25% above the upper end of the most probable estimate of K.

The only adequate method for deciding the proper size of K and its confidence limits is to repeat the experiment a number of times, within a fairly short interval of time, using a variety of models, and particularly obtaining the basic data by a variety of field methods. Unfortunately, if such intensive efforts had been made here they would have driven even more of the butterflies from the place of study, and excessive egress was already the principal difficulty in the present work. Therefore, continued research should be done to find additional methods for estimating density, particularly ones that disturb the population a minimum. The model recently proposed by Hanson (1968) might be helpful in this regard. In a nutshell, the best suggestion for lepidopterists, and zoologists in general, seems to be that they should use several good methods on each population studied and be prepared to accept errors up to 50% of the estimates made.

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